YK160  GLP-1 EIA Kit

I. Introduction

GLP-1 is a peptide hormone from the intestinal mucosa, which is produced from its precursor, proglucagon by post translational processing. The mammalian proglucagon\(^1\) is synthesized in the neuroendocrine L-cell of the intestine and the alpha-cells of the pancreas. It contains within its structure the sequences of glucagon and two glucagon-like peptides (GLP-1 and GLP-2) in tandem flanked at their amino and carboxyl termini by dibasic residues. GLP-1 is a 37 amino acids peptide and produced in the small intestine and in the pancreas in the human, in either C-terminal-amidated on glycine-extended form\(^2\)\(^3\).

GLP1 (7-36) amide and its receptor are present in several brain regions and may play a role in the physiological control of feeding\(^4\). Several reports have been presented as follows as to the biological activities of GLP-1. GLP-1 (7-37) and (7-36) amide is known as one of the most potent insulin secretagogues\(^5\).

GLP-1 (7-36) amide was supposed to improved glycemic control in patients with type 2 diabetes by increasing insulin secretion, by inhibiting glucagon secretion and by delaying gastric emptying rather than by altering extrapancreatic glucose metabolism\(^6\). Intravenous GLP-1(7-37) and (7-36)amide could normalize fasting hyperglycaemia in type 2 diabetic patients\(^7\). Hyperglycaemia during parenteral nutrition could be controlled by exogenous GLP-1, whereas the chronic therapy of type 2 diabetes required GLP-1 derivatives with longer duration of action\(^8\). Recombinant GLP-1 (7-36) amide was recently shown to cause significant weight loss in type 2 diabetics when administered for 6 weeks as a continuous subcutaneous infusion, 5-day treatment of hereby obese human subjects with GLP-1 at high doses by prandial subcutaneous infusion promptly slowed gastric emptying as a probable mechanism of action of increased satiety, decreased hunger and reduced food intake with an ensuing weight loss\(^9\).

A G-protein-coupled receptor, GPR120, which is abundantly expressed in intestine, functions as a receptor for unsaturated long-chain FFAs (free fatty acids). The stimulation of GPR120 by FFAs promotes the secretion of GLP-1 in vitro (measured by YK160, Yanaihara Institute Inc) and in vivo, and increases circulation insulin, indicate that GPR120-mediated GLP-1 secretion induced by dietary FFAs is important in the treatment of diabetes\(^10\).

All these approaches have shown remarkable efficacy in both experimental and clinical studies. The GLP-1-based therapy of type 2 diabetes, therefore, represents a new and attractive alternative\(^11\).

Yanaihara Institute Inc. developed a quantitative EIA kit with high specificity and sensitivity (detection limit 0.206ng/mL) for rat/mouse/human GLP-1 (YK160) as a useful tool for these necessaries.
YK160  GLP-1 EIA Kit

Contents
1) Antibody coated plate
2) GLP-1 standard
3) Labeled antigen
4) GLP-1 antibody
5) SA-HRP
6) Diluent for SA-HRP
7) Substrate buffer
8) OPD tablet
9) Stopping solution
10) Buffer solution
11) Washing solution (concentrated)
12) Adhesive foil

II. Characteristics

This EIA kit is used for quantitative determination of rat/mouse/human GLP-1 in plasma samples. The kit is characterized for sensitive quantification, high specificity and no influence with other components in plasma samples. GLP-1 standard is highly purified synthetic product.

< Specificity >
The EIA kit has high specificity to rat/mouse/human GLP-1 and shows cross reactivity neither rat/human/mouse glucagons, human glicentin nor rat/mouse/human GLP-2.

< Test Principle >
This EIA kit for determination of rat/mouse/human GLP-1 in plasma samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to GLP-1 (7-36) amide with biotin-avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG antibody. GLP-1 standard or samples, labeled antigen and GLP-1 antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptavidin (SA-HRP) are added to form HRP labeled streptavidin-biotinylated GLP-1-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of GLP-1 is calculated.
III. Performance Characteristics

Typical standard curve

<table>
<thead>
<tr>
<th>Add GLP-1 (ng/mL)</th>
<th>Observed (ng/mL)</th>
<th>Expected (ng/mL)</th>
<th>Recovery (%)</th>
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< Human plasma >

<table>
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<th>Add GLP-1 (ng/mL)</th>
<th>Observed (ng/mL)</th>
<th>Expected (ng/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<tr>
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Precision and reproducibility

Rat plasma
- Intra-assay CV (%) 5.36 ~ 6.60
- Inter-assay CV (%) 5.51 ~ 18.87

Human plasma
- Intra-assay CV (%) 4.69 ~ 10.67
- Inter-assay CV (%) 9.63 ~ 17.57

Assay range
0.206 ~ 50 ng/mL
IV. Stability and Storage

< Storage > Store all of the components at 2-8°C.
< Shelf life > 12 months from the date of manufacturing
The expiry date is described on the label of kit.
< Package > For 96 tests per one kit including standards

V. References


Update at July 13, 2005